

The Patterning Cascade Model and Expression of the Carabelli Feature in Humans: Differences
Between First and Second Molars and Correlation with Other Dental Traits

Honors Research Thesis

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Stephanie M. Moormann

Project Advisors: John P. Hunter & Debra Guatelli-Steinberg

Abstract

The Patterning Cascade Model of tooth development suggests that cusp formation follows a sequential developmental pattern. Enamel knots, specialized cells in a developing tooth from which cusps initiate, act as signaling centers in this developmental cascade. To form, an enamel knot must escape inhibition fields produced by other enamel knots. The Model predicts that the number and size of cusps will vary as a function of intercusp spacing relative to tooth size. The present research builds on work by Hunter et al. (2010) on the Carabelli feature, a common human dental character. These researchers found that the model predicted Carabelli feature expression in first molars. The focus of the present research is to determine if differences in the expression of Carabelli feature along the molar row can be predicted on the basis of the Model as well. These differences were assessed through a comparison of relative intercusp distance between first and second molars. Tooth sizes and intercusp distances were measured three times from dental casts of 380 individuals using a Hirox digital microscope. The results of this study show that the degree to which the Carabelli feature is expressed on first versus second molars is consistent with the Model's predictions. Other dental features were found to covary with the Carabelli feature. The results of this study suggest that dental traits are not independent of each other and instead develop in a dependent manner. This is important to consider because the degree to which dental traits covary must be taken into account when assessing evolutionary relationships based on these traits.

Table of Contents

Table of Contents	ii
Figures and Tables	iii
Acknowledgments	v
Introduction.....	1
<i>The Carabelli Feature</i>	1
<i>Tooth Development and the Patterning Cascade Model</i>	4
<i>The Model and First Molars</i>	6
<i>The Current Study</i>	7
Methodology	9
<i>Measurement Error</i>	11
Results.....	12
<i>First Molars and the Model</i>	12
<i>Second Molars and the Model</i>	14
<i>Comparison of First and Second Molars</i>	16
<i>The Hypocone and the Carabelli Feature</i>	16
<i>The Accessory Cusps and the Carabelli Feature</i>	18
Discussion.....	19
Conclusions.....	24
Figures and Tables (See iii)	(S26
References.....	45

Figures and Tables

Figure 1. A Diagram of the Four Cusps of the Upper Molar.....	26
Figure 2. Standardized Arizona State University Dental Plaques.....	27
Figure 3. A Diagram of the Accessory Cusps of the Upper Molar.....	28
Figure 4. Relative Intercusp Distance Observed in First Left Molars.....	29
Figure 5. Carabelli Feature Expression Observed in Regards to Relative Intercusp Distance for Left First Molars.....	30
Figure 6. Relative Intercusp Distance Observed in Left Second Molars.....	31
Figure 7. Carabelli Expression Observed in Regard to Relative Intercusp Distance for First and Second Molars.....	32
Figure 8. Differences Seen Between First and Second Molars in Mean Intercusp Distance and Absolute Tooth Size.....	33
Figure 9. Number of Molars per Carabelli Feature Grade across Hypocone Grades.....	33
Figure 10. Number of Molars per Hypocone Grade across Carabelli Feature Grades.....	34
Figure 11. Number of Molars per Carabelli Feature Grade across Hypocone Grades for First Molars.....	35
Figure 12. Number of Molars per Carabelli Feature Grade across Hypocone Grades for Second Molars.....	36
Figure 13. Number of Molars per Hypocone Grade across Carabelli Feature Grades for First Molars.....	37
Figure 14. Number of Molars per Hypocone Grade across Carabelli Feature Grades for Second Molars.....	38
Figure 15. Examples of the Accessory Cusps.....	39
Figure 16. Number of Accessory Cusps vs. Number of Teeth per each Carabelli Feature Grade.....	40

Figure 17. Number of Accessory Cusps vs. Number of Teeth per each Carabelli Grade for First Molars.....	41
Figure 18. Number of Accessory Cusps vs. Number of Teeth per each Carabelli Grade for Second Molars.....	42
Table 1. Measurement Error.....	43
Table 2. Comparisons of Mean Intercusp Distance and Tooth Size to Carabelli feature Expression.....	43
Table 3. Proportional Odds Logistic Regression of Carabelli Expression.....	44



Georg Carabelli, Edler von Lunkaszprie (1787-1842)

First Discover of Carabelli's Cusp

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Introduction

Carabelli's feature was first identified in 1842 by an Austrian dentist named Georg von Carabelli (Kraus, 2005). He described what he later named Carabelli's feature as a tubercle that was occasionally present on the mesiolingual surface of the permanent maxillary molars, noting that it was more frequently found on the first molars than the second or third (Kraus, 2005). Since this discovery, many studies throughout the past 169 years have devoted themselves to the Carabelli feature's descriptive morphology, its prevalence in different populations, its development, and finally its mode of inheritance. None of these studies, however, has sought to apply a morphogenetic model of development to Carabelli's feature, except that of Hunter et al. (2010). Hunter et al. (2010) successfully applied Salazar-Ciudad and Jernvall's (2002) patterning cascade model of tooth morphogenesis to Carabelli feature development on the upper first molars. The present study seeks to build on this research to further apply the Patterning Cascade Model to the other teeth along the molar row.

The Carabelli Feature

Carabelli's feature is found on the upper three molars on the mesiolingual surface (Alvesalo et al., 1975). Normally, the Carabelli feature is attached to the protocone, though the Carabelli feature can exist as an independent cusp (Figure 1). The size and degree of formation of the Carabelli feature varies considerably among individuals, from a small crease along the mesiolingual surface to a fully developed cusp comparable in size to the hypocone, metacone, protocone, and paracone. The variance in the Carabelli feature's size and form can be divided into eight graded categories ranging from the Carabelli feature's absence to a fully cuspal

Carabelli feature. These grades were established by Turner et al. (1991) and can be visualized on the Standardized Arizona State University (ASU) dental plaques (Figure 2). Carabelli's feature has an extensive nomenclature. Generally, molars expressing a Carabelli feature at ASU grades 1-4 are not considered to have a fully cuspal Carabelli feature, in this case the Carabelli feature is called the Carabelli trait. Molars expressing a Carabelli feature at ASU grades 5-7 are considered to be expressing a fully cuspal Carabelli feature; in this case the Carabelli feature is called the Carabelli cusp. For simplifying purposes this paper will be collectively calling Carabelli traits and cusps the Carabelli feature or just simply the Carabelli.

In addition to varying in its development, Carabelli expression varies from population to population. Fully cuspal forms are generally more frequent in European populations than in African or Native Americans populations (Alvesalo et al., 1975). Moreover, the expression of the Carabelli feature appears to vary between the sexes, occurring more frequently in males than in females (Alvesalo et al., 1975).

Early studies of the Carabelli have noted that the feature is frequently found bilaterally, with the expression of the feature appearing to be equivalent on the left and right side of the jaw (Alvesalo et al., 1975). However, it has further been noted that a certain amount of asymmetry has been observed within individuals (Alvesalo et al., 1975). Alvesalo et al. showed that there was a high correlation between the two sides of the jaw and suggested that there was a strong bilateral and symmetric occurrence of the Carabelli.

Regardless of symmetry, it has been observed in numerous studies, and even by Georg von Carabelli, that the Carabelli feature is more frequently observed in the first molars in than the second molars (Kraus, 2005). The observation, however, has been just that. It is a simple observation that other scholars thought important to note but never to examine closely. The

present study seeks to understand why there is such a difference in expression of the Carabelli feature seen between first and second molars and if the Patterning Cascade Model that Hunter et al. (2010) successfully applied to first molars can be equally applied to the second molars.

Previous studies have seen similar trends in the first and second molars. Reid et al. (1991) observed that expression of the Carabelli feature is associated with molar size, but that this trend is more apparent in first molars than second molars. This indicates that the second molars are controlled by the same mechanisms as the first molars. The development of second molars and first molars, however, both show that dental traits are dependent upon each other during development.

The study of Carabelli feature expression in second molars can additionally examine the Carabelli's correlation with other dental traits. The second molar is more likely than the first to have an underdeveloped hypocone or to lack it completely (Keene, 1968). The hypocone has classically been linked with Carabelli feature development with both considered to be connected with the general evolutionary trend in hominid dentition towards tooth size reduction and morphological simplification (Keene, 1968).

The evolutionary significance of the Carabelli is not fully understood. Reid et al. (1991) and many studies have indicated that an increase in crown size seen when the Carabelli is present as opposed to absent compensates for the loss of tooth material due to the reduction in size of second and third molars (Reid et al., 1991). Others claim that the low occurrence of the Carabelli feature is part of the current evolutionary trend towards a reduction of tooth size. In this case the Carabelli feature is associated with the simplification of the occlusal surface of the tooth (Reid et al., 1991). Along these lines Scott (1979) theorizes that the decrease in appearance

of the Carabelli feature is the result of molar size reduction and loss of other maxillary molar characteristics.

Regardless of what the evolutionary implications of the Carabelli feature and the hypocone may be, Scott clearly shows that the two traits are linked. Previous research indicates that second molars in addition to having less frequent expression of the hypocone also have less frequent express of the Carabelli feature. This fact reduces the sample size of available molars for testing hypothesis related to the Carabelli feature in second molars.

Second molars are not alone in showing a linkage of other cusps with the Carabelli feature. Scott (1978) clearly indicates that the protostylid covaries with the Carabelli feature (Scott, 1978). The present study has found that the Carabelli feature covaries with the mesial accessory tubercle (MAT), the protoconule, the mesial paracone tubercle (MPT), the Metaconule (ML), the lingual paracone tubercle (LPT), and Cusp 5 (C5), collectively called the accessory cusps for the purposes of this study (Figure 3). This observation indicates that there may be a common genetic control in the expression of cusps along the crown.

Tooth Development and the Patterning Cascade Model

One of the major goals of the present study is to apply the Patterning Cascade Model (Salazar-Ciudad et al., 2002) to the upper second molars and to determine if the differences seen between first and second molars can be explained by the Patterning Cascade Model. To fully understand the implications of this application it is important to understand tooth development and its relationship to the Model.

During development, enamel knots are the precursors to tooth cusps. Enamel knots are zones of post-mitotic cells of the dental epithelium that produce epithelial growth factors

(Vaahtokari et al., 1996). Enamel knots initiate the formation of cusps by directing the growth of dental epithelium downward and away from the future cusp tips (Vaahtokari et al., 1996). In order for a new cusp to form, a precursor enamel knot must first form in the dental epithelium prior to mineralization (Vaahtokari et al., 1996). Because enamel knots cannot form within the inhibitory fields produced by the other enamel knots, the spacing of enamel knots and the size of inhibition fields strongly influence final tooth shape (Salazar-Ciudad et al., 2002). Because tooth shape development occurs during a defined time period prior to root formation, the window of opportunity for an enamel knot to form is limited (Salazar-Ciudad et al., 2002). The tooth's final size, as well as its shape, is limited due to this time constraint (Salazar-Ciudad et al., 2002).

The Model assumes a starting point of identical epithelial cells lying above a set of identical mesenchymal cells (Salazar-Ciudad et al., 2002). These mesenchymal cells can respond to one of two diffusible signaling molecules that affect the growth of the tooth germ line: an activator or an inhibitor (Salazar-Ciudad et al., 2002). The activator will induce cellular differentiation while the inhibitor will repress growth, each of which will be present in different concentration peaks (Salazar-Ciudad et al., 2002).

The Model depicts tooth development from the cap stage to the early bell stage (Salazar-Ciudad et al., 2002). Initially, all of the epithelial cells secrete activator molecules and when local activator molecule concentrations exceed a set threshold, the epithelial cells differentiate irreversibly into non-dividing knot cells (Salazar-Ciudad et al., 2002). These newly formed knot cells start to secrete inhibitor molecules at a rate equal to local activator concentration which counteracts activator secretion, preventing surrounding cells from differentiating into knot cells (Salazar-Ciudad et al., 2002). After these occurrences, the remaining epithelium continues to grow folding into the mesenchyme, and in the process, leaving the knots isolated in the tips of

the forming cusps (Salazar-Ciudad et al., 2002). The mesenchyme produces a localized lateral expansion-molecule that affect the sharpness of the cusps and thus the effective distance at which new knots can form (Salazar-Ciudad et al., 2002). Cusp sharpness and the growth of the tooth borders affect the overall shape of the knots which will affect the spatial distribution of any new enamel knot formation (Salazar-Ciudad et al., 2002). The implications of this model are that new enamel knot formation depends on the already existent morphology of the tooth, not only on the spatial distribution of the activator and inhibitor (Salazar-Ciudad et al., 2002).

Based on the implications of the Model, the spatial patterning of the cusps and the overall size of the molar can be seen as consequences of developmental events. The Patterning Cascade Model predicts that the number and size of cusps expressed will vary in relation to intercuspal spacing relative to tooth size. The Carabelli feature will tend to form if the main cusps are closely spaced for their given tooth size. Generally, larger teeth or teeth with a smaller intercuspal distance will have a greater probability of forming a Carabelli feature.

The Model and First Molars

This research builds on work by Hunter et al. (2010) that found that the Patterning Cascade Model accurately predicted Carabelli feature expression in first molars both across and within individuals. The data generated by Hunter et al. (2010) showed that, independent of genotype, small variations in the developmental timing or spacing of enamel knots can influence the number and pattern of cusps (Hunter et al., 2010). Hunter et al. (2010) validated the Patterning Cascade Model by showing that the Carabelli feature was more likely to be present in large teeth with smaller intercuspal distances relative to tooth size (Hunter et al., 2010). These relationships seen in the fully developed tooth are indicative of conditions in the developing

tooth that provided a greater opportunity for a new enamel knot to form beyond the inhibitory fields of the earlier-formed enamel knots (Hunter et al., 2010).

Hunter et al. (2010) placed a greater importance on the differences in intercuspal spacing in molars that lacked a Carabelli feature than molars that had a fully developed Carabelli feature, indicating that new enamel knots are more likely to form when earlier-forming enamel knots are closely spaced as opposed to forming when tooth development lasts for a longer time period (Hunter et al., 2010). However, the findings in Hunter et al. (2010) indicate that the interaction between enamel knot spacing and the duration of crown morphogenesis is what best reflects the developmental events leading to the Carabelli feature (Hunter et al., 2010). This was identified through an assessment of Carabelli expression against the intercuspal spacing relative to tooth size (Hunter et al., 2010).

The Current Study

The present study builds on the work and conclusions of Hunter et al. (2010) with two major goals. The first is to determine if the model can account for Carabelli feature expression in second molars. The second is to determine if the model can account for known differences in Carabelli feature expression in first and second molars. The Model predicts that the smaller the inter-cuspal distance is relative to tooth size, the greater the probability that a Carabelli feature will form in second and first molars. A negative relationship between relative intercuspal distance and Carabelli feature expression should be seen if the Model's predictions are met. To determine if the Patterning Cascade Model can account for the differences, the model predicts that the differences in Carabelli feature expression between the two tooth types will be associated with differences in their average relative intercuspal distances. First molars will either have a larger

tooth size or a smaller absolute inter-cusp distance, or both, to account for a higher frequency of Carabelli feature expression in first as compared to second molars.

Further, the present study will examine correlates of Carabelli feature expression seen in second and first molars to determine if the Carabelli feature expression is related to other dental features. Through observation of the data set, it has been noted that the Carabelli feature development is correlated with hypocone development and that both the hypocone and Carabelli feature are observed less frequently in second molars as opposed to first. If the hypocone and the Carabelli feature are contingent upon each other then the lack of expression of one should result in the lack of expression of the other. Moreover, the appearance of accessory cusps is noted to increase in the presence of the Carabelli feature. If the presence of accessory cusps and the Carabelli feature are correlated then a statistically significant increase in frequency of accessory cusps should be seen as the Carabelli feature become more fully developed.

The results of this study can be applied to numerous anthropological and evolutionary studies that routinely use dental traits to determine the biological relationships between two species or within species, as teeth are more likely than any other part of the body to fossilize. If these results are consistent with the results seen in Hunter et al. (2010) they would further challenge an underlying assumption that is utilized in many dental studies: that dental traits are independent of each other (Salazar-Ciudad et al., 2002). If the Patterning Cascade model applies to human teeth then this assumption will no longer be accurate. Further research from this study may be applied to other dental traits in humans, hominid species, and other mammals.

Methodology

The sample consisted of 296 left first molars, 150 left second molars, 295 right first molars, and 140 right second molars. Each analysis made use of a different subset of the complete sample, as each analysis was looking to indentify a unique trait that sometimes could only be associated with a subset of the data. Each molar was studied using a Hirox digital microscope that magnified the sample to a field of 15 x 15 mm. The casts were oriented in the occlusal plane defined by the tips of the cusp. The tooth areas and intercusp distances were measured three times on both first and second molars from dental casts from the Menegaz-Bock dental cast collection. This population came from Dayton, Ohio where the casts originated from an orthodontist. Three different measurements taken per tooth were averaged together for analysis in an effort to obtain more accurate data and minimize measurement error.

The Carabelli feature was graded in a standard format based on the Standardized Arizona State University (ASU) dental plaques (Turner et al., 1991; Figure 2). In this scoring system the Carabelli feature was graded on a scale of 0 to 7, with 0 representing molars lacking a Carabelli feature and 7 representing molars with a fully developed Carabelli feature. In an additional scheme, the Carabelli feature was divided into 3 categories: absent consisting of ASU grade 0, slight consisting of ASU grades 1-4 (also called the Carabelli trait), and present consisting of ASU grades 5-7 (also called the Carabelli cusp).

The hypocone was graded in a manner similar to that of the Carabelli feature. The hypocone grades were based on the standard format based on the Standardized Arizona State University (ASU) dental plaques (Turner et al., 1991). In this scoring mechanism the hypocone was graded on a scale of 0 to 5, with 0 representing molars that lacked a hypocone, and 5 representing molars with a fully developed hypocone.

The accessory cusps: MAT, MPT, ML, the protoconule, LPT, and C5 (Figure 3) were identified via observation of molars under a Hirox digital microscope. The accessory cusps were identified individually as being either present or absent on the molars. An accessory cusp was identified as being present if there was a clear protuberance on the occlusal surface of the molar at the classical position attributed to each accessory cusp.

The data were separated into four categories: left first molars, left second molars, right first molars, and right second molars. This initial sorting was done to avoid autocorrelation of right and left molars from the same individual and to separate the effects of the first and second molars from each other.

In first and second molars the relative intercusp distance was assessed with a simple linear regression (Kaps, et al., 2004) comparing absolute tooth size to the mean intercusp distance. Simple linear regression (Kaps, et al., 2004) comparing the relative intercusp distance and the absolute area of the Carabelli feature were used to examine the Carabelli feature's size in relation to the Model's predictions. Differences seen between and within first and second molars were assessed via comparisons of mean intercusp distance and absolute tooth size using a Student's t-test.

A simple linear regression (Kaps, et al., 2004) was used to observe the trends seen between the hypocone and the Carabelli feature comparing the respective grade of the Carabelli to the hypocone per molar. The data were separated as before into four categories: left first molars, left second molars, right first molars, and right second molars for reasons already stated. The data was examined within the complete data set and in each subset of the data set.

Proportional odds logistic regression was used to observe the trends seen between the presence of the accessory cusps and the Carabelli feature. The Proportional odds logistic

regression compared the number of accessory cusps present per molar to the ASU grade of the Carabelli feature seen on the respective molar. Again the data was separated into four categories: left first molars, left second molars, right first molars, and right second molars. Further the data was examined within subset of the complete data set. The number of accessory cusps seen per each molar was labeled as the response variable. Significance of the whole model is assessed with a likelihood ratio test in comparison with the null model.

Measurement Error

Each tooth was measured three unique times and the data were averaged together. Measurement error associated with the measurement techniques was assessed through Model II ANOVA. The relative measurement error (ME) was calculated as a percentage of the total variation among individuals and within individuals. Model II ANOVA was used to partition the total variance. The total sum of squared deviations from the grand mean (SS_{total}) was divided into among-individual (SS_{among}) and within-individual (SS_{within}) components (Yezerinac, et al., 1992). Mean squared deviations (MS) were equal to the SS divided by the degrees of freedom seen in the sample (Yezerinac, et al., 1992). The MS for each of the components was used to calculate the variance components (Yezerinac, et al., 1992). Among-individual variance (S^2_{among}) was calculated using the following formula: $S^2_{among} = \frac{MS_{among} - MS_{within}}{m}$ (Yezerinac, et al., 1992), where m is the number of repeated measurements. Within-individual variance (S^2_{within}) was set to be equal to SS_{within} (Yezerinac, et al., 1992). Percentage measurement error was calculated using the following formula: $\%ME = \frac{S^2_{within}}{S^2_{within} + S^2_{among}} \times 100$ (Yezerinac, et al., 1992). Model II ANOVA was used instead of Model I ANOVA because the error in the measurements should be random and not fixed.

The measurement error can be seen in Table 1. The measurement error for intercuspal distance is between 3.5%-24.9%, and measurement error for tooth area and Carabelli feature area falls between this range. The larger measurement error seen for intercuspal distances may be due to the smaller magnitude of these distances. The given measurement error should be random and thus it should obstruct correlations that present in the data without creating additional correlations.

Results

First Molars and the Model

The complete sample of upper first molars clearly falls within the parameters of the Patterning Cascade Model and further exemplifies the trends described by Hunter et al. (2010). The findings of Hunter et al. (2010) supported the model's predictions for Carabelli feature formation and suggest that the timing or spacing of enamel knots can influence cusp patterning independent of genotype.

The complete sample of first molars mirrors the sample of first molars used in Hunter et al. (2010). In both samples, first molars with a Carabelli feature possess lower mean intercuspal distances than first molars without a Carabelli feature, which by itself is indicative of the Patterning Cascade Model's predictions. Additionally, first molars with a Carabelli feature express a lower mean intercuspal distance relative to tooth size as compared to first molars without a Carabelli feature. Molars that expressed a slight Carabelli feature span a larger range of values of intercuspal distance relative to absolute tooth size than molars lacking or expressing a more developed Carabelli feature. The value that the molars with slight Carabelli expression span

overlap the ranges of the other two groups (Figure 4). Consistent with Hunter et al. (2010), left and right molars were separated to avoid correlation between antimeric pairs.

Mean intercuspal distance and square root of the tooth area were explored individually in relation to Carabelli feature expression for left first molars. Just as before the molars were divided into three categories in regards to Carabelli feature expression: present (ASU grade 5-7), slight (ASU grade 1-4), and absent (ASU grade 0). Molars included in the present and absent categories represented each extreme end of the spectrum. The molars at each extreme end of the spectrum showed opposite patterns in regards to mean intercuspal distance and absolute tooth size (Table 2). Molars with a fully developed Carabelli feature and lacking a Carabelli feature were consistent with each other in regards to length. The two extremes differed however, in their mean intercuspal distance. Molars with a fully developed Carabelli feature have a smaller mean intercuspal distance compared to molars lacking a Carabelli feature. These data replicate the observations seen in Hunter et al. (2010) with a larger sample size. Further, these data show that the Carabelli feature development seen in the first molars in this sample is due to differences in relative intercuspal distance and not tooth size, just as in Hunter et al. (2010).

First molars were further tested to see if Carabelli size was correlated with relative intercuspal distance. Simple linear regression was used to examine the functional relationship of Carabelli feature expression to relative intercuspal distance. Only teeth that had a measurable Carabelli cusp (ASU grades 5-7) were used in this sample. For these upper first molars, Carabelli feature expression was seen to be negatively correlated with relative inter-cuspal distance in first molars ($t=18.2499$, $p<0.001$, $df=584$; see Figure 5). These results are not surprising as they suggest the same conclusions as Hunter et al. (2010). It can be seen that differences in cuspal spacing can affect the Carabelli development and size.

Second Molars and the Model

On average second molars are known to be smaller in size and absolute tooth area than first molars. This difference in size may be the largest factor to consider when evaluating the differences seen in Carabelli feature expression between the first and second molars, an observation even noted by Georg von Carabelli (Kraus, 2005). A major objective of this study was to determine if the Patterning Cascade Model would be able to accurately predict the expression of the Carabelli feature for the upper second molars as it has already been shown to predict the expression of the Carabelli feature for upper first molars.

Second molars varied greatly from first molars in their expression of the Carabelli feature. Generally it is more common for a second molar that expresses the Carabelli feature to fall into the lower ASU grades than the fully cuspal forms. Further a greater proportion of second molars lacked a hypocone. This lack of a hypocone expression is consistent with a lack of the Carabelli feature expression in second molars (Reid et al., 1991). Due to the correlation of the hypocone with the Carabelli feature it can be seen that second molars lack the hypocone also tend to lack the Carabelli feature. Since second molars have a greater frequency of underdeveloped hypocones, they have smaller sample size of molars with a Carabelli feature than first molars. Further, the noticeably smaller size of second molars could be playing a major role in the lower number of molars expressing Carabelli features.

The trends seen in the complete sample of upper second molars parallel the trends seen in the upper first molars, with a noticeable difference. There were no fully cuspal forms of the Carabelli feature observed within left second molars and right second molars did contain significant amount of fully cuspal forms of the Carabelli feature. It should be noted that second molars that expressed a slight Carabelli feature possess lower mean intercuspal distances than

second molars that lack a Carabelli feature, which supports the Patterning Cascade Model's predictions. Additionally, second molars with a slight Carabelli feature express a lower mean intercuspal distance relative to tooth size as compared to second molars without a Carabelli feature (Figure 6).

Further, mean intercuspal distance and square root of the tooth area were explored individually in relation to Carabelli feature expression for left second molars. Again, molars at each extreme end of the spectrum for left second molars were used (Table 2). However, the top extreme represented molars in Carabelli feature grades 4-6 instead of Carabelli feature grades 5-7 because the left second molars lacked molars in Carabelli feature grade 7. Molars lacking a Carabelli feature represented the bottom extreme. The two extremes have virtually identical mean inter-cuspal distances; they differed however in their mean square root tooth area. This suggests an opposite trend than what was observed in the first molars. However, it should be noted that both data sets are consistent with the Patterning Cascade Model in regards to intercuspal distance in relation to the tooth area. Examined together, both of the molar types indicate that it is the ratio between the intercuspal distance and absolute tooth area that is an indicator of Carabelli feature formation. Additionally, this finding is consistent with the main difference seen in first and second molar Carabelli feature expression.

The left second molars were further examined to see if the Carabelli feature's size was correlated with relative intercuspal distance. Simple linear regression was used to examine the functional relationship of the Carabelli feature expression to relative intercuspal distance. Only teeth that had a measurable Carabelli feature were used in this sample. For these upper second molars, Carabelli feature expression was not seen to be significantly correlated with relative intercuspal distance ($t = 1.279$, $p = 0.2020$, $df = 280$; Figure 7). This lack of statistical significance

may be explained by the fact that the sample lacks teeth without fully formed Carabelli features, which itself, is meaningful.

Comparison of First and Second Molars

It has already been clearly observed, in previous studies (Reid et al., 1991), that upper first and upper second molars have different probabilities of expressing a Carabelli feature and different frequencies of molars that fall into each cuspal category. A major goal of this study was to see if the Patterning Cascade Model could account for these differences in Carabelli feature expression between first and second molars. First molars show a greater frequency of Carabelli feature expression. Of 591 first molars, 254 expressed the Carabelli trait (grades 1-4), and 17 expressed a well-developed Carabelli cusp (grades 5-7). Of 290 second molars, 71 expressed the Carabelli trait (grades 1-4), and 7 expressed a fully developed Carabelli feature (grades 5-7).

When the average intercusp distance and absolute tooth size are compared individually between first and second molars, it can be seen that first and second molars have similar absolute intercusp distances ($t = 2.95$, $p = 0.0037$, $df = 131$; see Figure 8). However, first and second molars are noticeably different in absolute tooth size ($t = 5.5297$, $p = 0.0001$, $df = 131$; see Figure 8). In accordance with the model, the smaller the intercusp distance relative to tooth size the greater the probability that the Carabelli feature will form. Differences in size at the same intercusp spacing may account for the greater frequency of Carabelli feature expression in first molars as compared to second molars.

The Hypocone and the Carabelli Feature

The hypocone has classically been linked with Carabelli feature development, with both considered to be connected with the general evolutionary trend in hominid dentition towards

tooth size reduction and morphological simplification (Scott, 1979). Within the Dayton population it can be seen that when the hypocone is not developed there is little if any observation of a Carabelli feature. When the hypocone's grade is zero, 94% of the molars in the Dayton sample had a Carabelli grade of zero as seen in Figure 9. However, when the hypocone's grade is five, no one Carabelli feature grade is dominant or even over 50% of the sample. The trends seen for Carabelli feature development throughout each hypocone grade indicate a decreasing negative trend from hypocone grade 0 to hypocone grade 5. Thus the more developed the hypocone is, the more likely it is that the Carabelli feature will be fully developed as well.

The reverse relationship, however, is not true as a range of hypocone development can be seen when the Carabelli feature is absent (Figure 10), indicating that the development of the hypocone is not dependent on the development of the Carabelli feature. Figure 10 additionally indicates the same trend seen in Figure 9, that a highly developed hypocone is correlated with a highly developed Carabelli feature.

The individual trends seen within the first and second molars when comparing the Carabelli feature grades against the hypocone grades, and when comparing the hypocone grades against the Carabelli feature grades, mirror the trends seen within the entire sample. When examined individually, however, first molars have a greater percentage of highly developed hypocones as compared to second molars (Figure 11-14). It has been observed from the frequency of occurrence that second molars are more likely to have an underdeveloped hypocone or lack a hypocone than first molars (Scott, 1979). Second molars on average are smaller than first molars and this difference may account for lower percentage of developed hypocones and Carabelli features seen in Figure 12 and Figure 14. The more highly expressed hypocone and

Carabelli feature grades seen in first molars follow the same trends observed in the complete data set: a smaller intercusp distance relative to overall tooth size produces a molar with more cusps.

The Accessory Cusps and the Carabelli Feature

As the Carabelli feature's development becomes more pronounced, the prevalence of six accessory cusps (occlusal tubercles) increases in frequency. Further, these tubercles were more frequent when the Carabelli feature was present as opposed to absent. Three of these occlusal tubercles are on the mesial ridge of the upper molars. The first is the mesial accessory tubercle (MAT), located on the mesial marginal ridge between the mesial paracone tubercle and protoconule (Scott, et al., 1997). The mesial paracone tubercle (MPT) is expressed as an independent part of the mesial accessory ridge of the paracone (Scott, et al., 1997). The final tubercle on the mesial accessory ridge, the protoconule, is located on the hypertrophied mesial accessory ridge of the protocone with an independent cusp tip (Scott, et al., 1997). The metaconule (ML) is not part of the ridge complex but is adjacent to it (Scott, et al., 1997). The metaconule is an occlusal tubercle between the protocone and the metacone (Scott, et al., 1997). The two additional cusps are located near the distal ridge. The lingual paracone tubercle (LPT) is not associated with the ridge complex; it is lingual and mesial to the terminus of the median ridge of the paracone (Scott, et al., 1997). It is important to note that this feature sometimes merges with either the mesial paracone tubercle or mesial accessory tubercle (Scott, et al., 1997). Cusp 5 (C5) is an occlusal tubercle on the distal marginal ridge of the upper molars (Scott, et al., 1997). It is usually rounded or conical but will appear triangular in more pronounced expressions (Scott, et al., 1997). The cusp is more common on the upper first molar but exhibits more pronounced forms of expression on second and third molars (Scott, et al., 1997). The locations of

these cusps can be seen in Figure 3 and examples of these cusps from the sample can be seen in Figure 15.

When comparing the expression of accessory cusps to the development of the Carabelli feature it is apparent that the more developed the Carabelli feature is the greater the frequency that an accessory cusp will form. As indicated in Figure 16 the overall number of accessory cusps that are present at higher Carabelli grades is notably higher when compared to the overall number of accessory cusps that are present at Carabelli grade 0.

As indicated by the proportional logistic regression model, overall the probability of forming extra cusps increases as the ASU grade increases (Table 3). In left first molars when the ASU grade changes by a score of 1 the probability of developing an accessory cusp increases by 1.25:1. In right first molars, when the ASU grade changes by a score of 1, the odds of developing an accessory cusp increases by 1.114:1. In left second molars when the ASU grade changes by a score of 1 the odds of developing an accessory cusp increases by 1.284:1.

When compared against each other, first molars show a greater incidence of accessory cusp expression compared to second molars (Figure 17 and Figure 18). This can be seen as a correlate of the first molars tendency to express more pronounced Carabelli features. Due to this correlation it can be theorized that the same factors that promote Carabelli expression additionally affect accessory cusp expression. It is clear the molars with large areas and smaller intercusp distances promote accessory cusp expression.

Discussion

The results from this study lend further support to the Patterning Cascade Model as the Model's predictions hold true for both first molars and for differences seen between first and

second molars. In accordance with the model it can be seen that the smaller the inter-cusp distance relative to tooth size, the greater the probability that the Carabelli feature will form. Differences in size at the same intercuspal spacing may account for the great frequency of Carabelli feature expression in first molars as compared to second molars. Additionally, the Model can account for the expression patterns seen of hypocone and accessory cusp expression as each of these expression patterns is correlated with Carabelli feature expression in first upper and second upper molars.

The complete sample of upper first molars clearly falls within the predictions of the Patterning Cascade Model and further extends the trends found by Hunter et al. 2010. Among first molars, smaller inter-cusp distance implies that enamel knots were closely spaced together during tooth development and thus created smaller inhibitory fields. Larger tooth size implies that teeth grow for a longer period of time or at a faster rate, either of which would create opportunities for an enamel knot to form outside of the inhibitory fields during tooth development (assuming that intercuspal distance does not also increase with tooth size). Further, molars at each extreme of Carabelli expression (ASU grades 5-7 and ASU grade 0) were almost identical with respect to absolute tooth size but differed significantly in regards to mean intercuspal distance. These data indicate that Carabelli feature development is not auto-correlated with absolute tooth size or mean intercuspal distance. The variance in Carabelli feature expression seen in first molars is instead due to differences in relative intercuspal distance and not tooth size alone, as predicted by the Patterning Cascade Model.

Second Molars, unlike first molars, do not express a large frequency of molars with Carabelli grades 5, 6, and 7. However, the trend seen in the complete set of upper second molars does parallel the trend seen in the upper first molars. In both first and second molars a

comparatively lower relative intercuspal distance indicates a greater probability of Carabelli feature expression. Second molars, however, have a smaller number of fully cuspal forms of the Carabelli feature (ASU grades 6-7). This observation may account for the difference in statistical significance seen when applying the same analytical tests to the second molars as those applied to the first molars. Additionally, the sample size of second molars was reduced as compared to the first molars, and this smaller sample size could be affecting the trend observed.

The second molars varied greatly from the first molars in the number of teeth that expressed a Carabelli feature. Generally it was more common for a second molar that expresses Carabelli features to fall into the lower ASU grades than the fully cuspal forms. Lack of Carabelli development in second molars is also associated with either reduced hypocone expression or hypocone absence. Thus Carabelli feature expression is dependent on hypocone expression.

The major difference between first and second molars is well known to be size. Second molars are known to be smaller than first molars, and the differences in Carabelli feature expression between first and second molars may be attributed to this. In fact, the major difference between the first and second molars is mirrored when the two extremes of the second molars are compared (ASU grades 4-6 and ASU grade 0). The two extremes have virtually identical mean intercuspal distance, they differed however in their mean square root tooth area, an opposite trend than what was observed in the first molars but identical to the trend seen when second and first molars are compared against each other. This indicates the absolute tooth size is driving the difference in Carabelli feature expression between first and second molars. If a molar is not large enough to accommodate a new enamel knot, it will not form regardless of the size of the mean intercuspal distance. Perhaps this is an indication of a threshold in absolute tooth size

that must be met in order for a Carabelli feature to form. It can be postulated that after this threshold is reached then mean intercusp distance will be the main determinant of Carabelli feature development and expression.

This observation does not contradict the Patterning Cascade Model, however, nor does it contradict the observations seen within the first molars. Both data sets are consistent with the Patterning Cascade Model in regards to intercusp distance in relation to tooth area. When examined together, both of the first and second molars indicate that it is the ratio between the intercusp distance and absolute tooth area that is an indicator of Carabelli feature formation.

Within the complete data set of both first and second molars it can be seen that when the hypocone is not developed there is little if any chance that a Carabelli feature will form. Additionally, the more developed the hypocone is, the more likely it is that the Carabelli feature will be fully developed as well. The reverse relationship, however, is not true as a range of hypocone development can be seen when the Carabelli feature is absent, indicating that the development of the hypocone is not dependent on the development of the Carabelli feature. First molars have a larger percentage of highly developed hypocones as compared to second molars.

It can be clearly seen that the more developed the Carabelli feature is the greater the probability that an accessory cusp will form. This trend, however, only seems to be true concerning Carabelli feature grades 0 – 6. At Carabelli feature grade 7 the presence of accessory cusps is less likely. Two possible hypotheses can account for this trend. First it can be speculated that this phenomenon occurs because the Carabelli feature is fully developed and thus is occupying more tooth area and producing a larger inhibitory field. Or second, the lack of significance for ASU grade 7 may be a result of the small sample of teeth of ASU grade 7 seen in the sample. If the second hypothesis is correct a larger sample size will show that Carabelli

grades 6 and 7 behave in the same manner as Carabelli grades 0-5. Regardless, the overall trend seen when comparing Carabelli expression to accessory cusp expression is positive. Further, when compared against each other, first molars show a greater incidence of accessory cusp expression compared to second molars. This can be seen as a correlate of the first molars' tendency to express more pronounced Carabelli features. Thus, it can be theorized that the same factors that promote Carabelli expression additionally affect accessory cusp expression.

The correlation of the expression of the accessory cusps and the hypocone with Carabelli feature expression is indicative of control by the same mechanism(s). These correlations show that the same mechanisms control tooth development as predicted by the Patterning Cascade Model. Furthermore, there may be common genetic controls underlying the potential to develop the major cusps and additional cusps such as the Carabelli feature and the accessory cusps.

The results of this study can be applied to numerous anthropological and evolutionary studies that routinely use dental traits to determine the biological relationships between two species or within species, as teeth are more likely than any other part of the body to fossilize. Along those lines, these results can challenge an underlying assumption that is utilized in many dental studies and classifications of species: that dental traits are independent of each other (Salazar-Ciudad et al., 2002; Hunter et al., 2010). As Kangas et al. (2004) has indicated, previous studies in evolution of mammals have asserted that developmentally most dental characters are non-independent of each other. If the Patterning Cascade model applies to human teeth then the assumption that dental traits develop independent of each other will no longer be accurate. In light of this, the research from this study may be applied to other dental traits in humans, hominid species, and other mammals.

Future studies, should concern themselves with identifying populations of individuals that characteristically have more cuspal forms of the Carabelli feature of their second molars. A population that contains fully cuspal forms of the Carabelli feature may show the same level of statistical significance from second molars as seen in first molars when second molars are examined within the model. Or conversely, if no such population or sample can be found, the lack of fully cuspal forms of the Carabelli feature on second molars would speak volumes about the differences in development between second and first molars. Additionally, the correlation between the Carabelli feature and the accessory cusps should be further investigated through examining the graded development of each accessory cusp against the graded development of the Carabelli feature in much the same way as the hypocone was compared to the Carabelli feature in this study.

Conclusions

The results of this study lend support to the Patterning Cascade Model as the Model can accurately predict the expression patterns of the Carabelli feature in upper first molars just as the research done in Hunter et al. (2010) did. Furthermore, while the Patterning Cascade Model can account for the differences seen between upper first and second molars; mainly it shows the second molars are markedly smaller than first molars and that their size hinders the expression of the Carabelli feature. Moreover, the expression of the Carabelli feature is reduced within second molars in association with a larger frequency of these molars lacking the hypocone, which has been shown to be correlated with the Carabelli feature (Scott, 1979). The results of the study show that the Carabelli feature's presence may be partially contingent on the hypocone's presence. Second molars, however, are not alone in the fact that other dental features on the occlusal surface seem to be correlated with the Carabelli feature. Both second and first molars

show a correlation of accessory cusp with Carabelli feature expression, showing a greater percentage of accessory cusp expression as the Carabelli feature becomes more developed. All of the data generated by this study shows that these dental traits do not develop independently from each other but are instead interrelated.

Figures and Tables

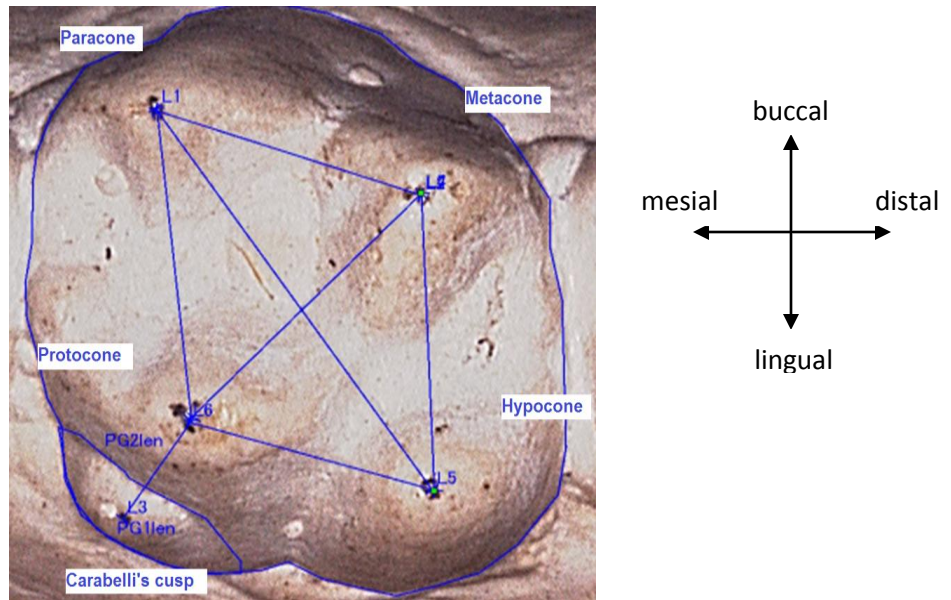


Figure 1. A Diagram of the Four Cusps of the Upper Molar. A molar with the four major cusps labeled: protocone, metacone, hypocone, and paracone. Additionally this molar has the Carabelli's feature. The Carabelli feature is expressed next to the protocone, as seen in the Figure, when present. Inter-cusp distances are the blue lines between the cusp tips.



Figure 2. Standardized Arizona State University Dental Plaques. A scale of 0 to 7 is used, where 0 indicates the absence of the Carabelli feature and 7 indicates a fully developed Carabelli feature (Turner et al., 1991). Photo Credit: John P. Hunter.

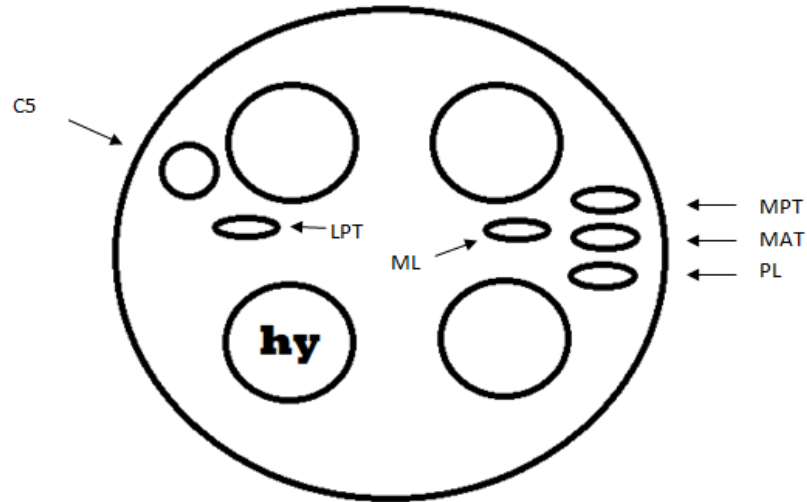


Figure 3. A Diagram of the Accessory Cusps of the Upper Molar. (upper molar accessory marginal and occlusal tubercles). hy: hypocone. MPT (mesial paracone tubercle), MAT (mesial accessory tubercle), PL (protoconule), ML (metaconule), LPT (lingual paracone tubercle), and C5 (cusp 5).

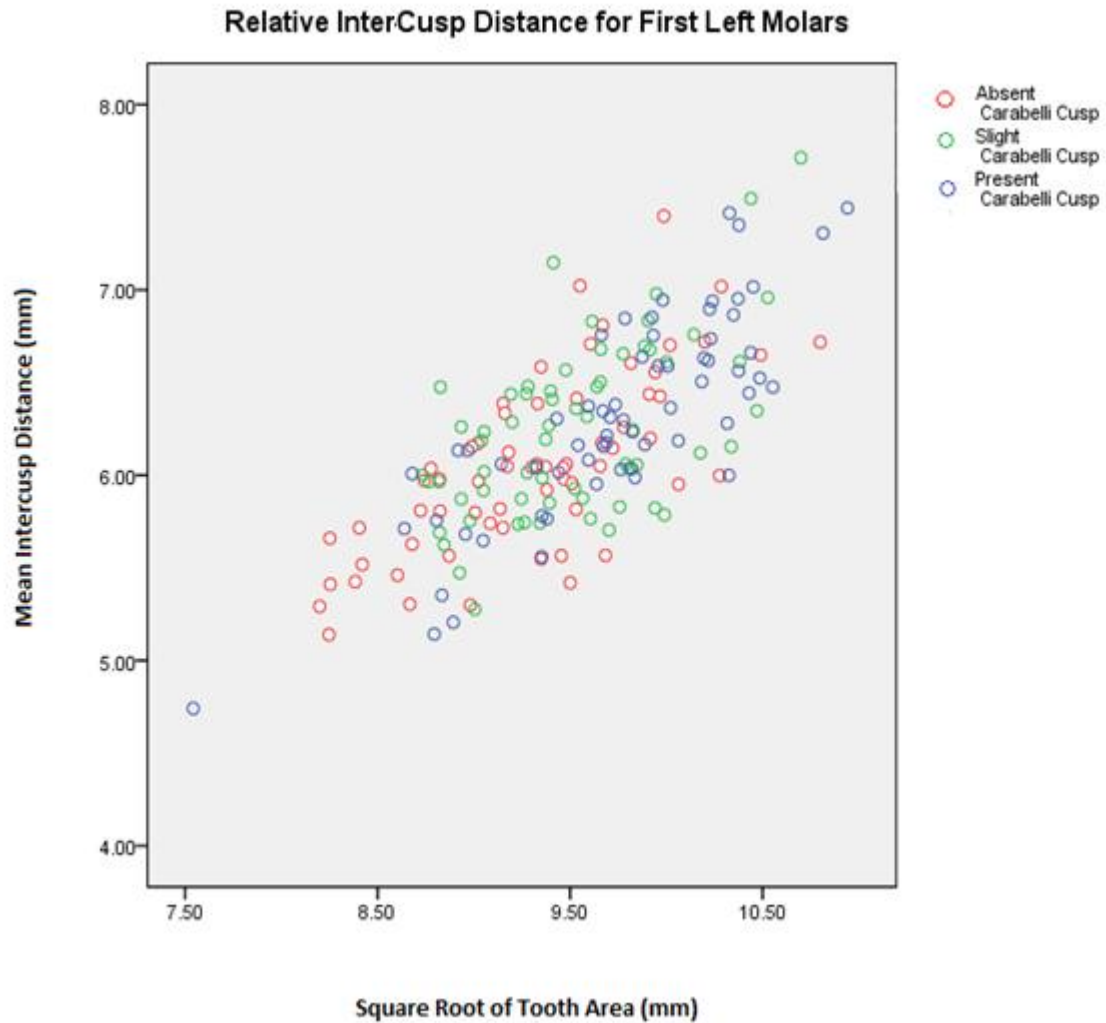


Figure 4. Relative Intercusp Distance Observed in First Left Molars. The square root of tooth area in mm (absolute tooth area) is graphed against the mean intercusp distance in mm. The molars are divided into three categories: absent consisting of ASU grade 0, slight consisting of ASU grades 1-4, and present consisting of ASU grades 5-7.

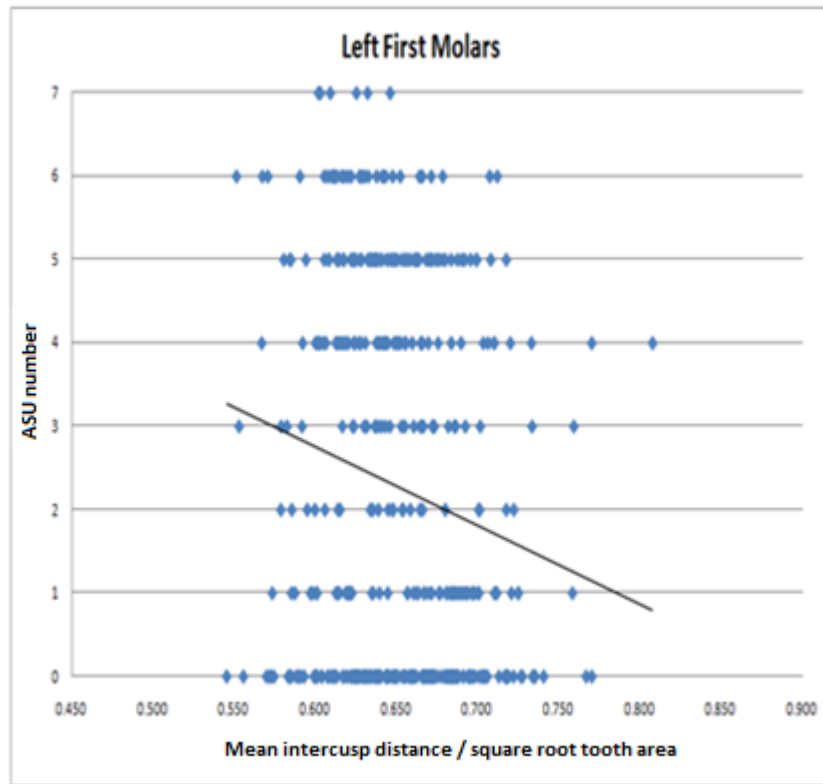


Figure 5. Carabelli Feature Expression Observed in Regards to Relative Intercusp Distance for Left First Molars. Carabelli feature expression (Carabelli grade) is negatively correlated with relative intercusp distance in first molars ($\tau=18.2499$, $p<0.001$, $df= 584$).

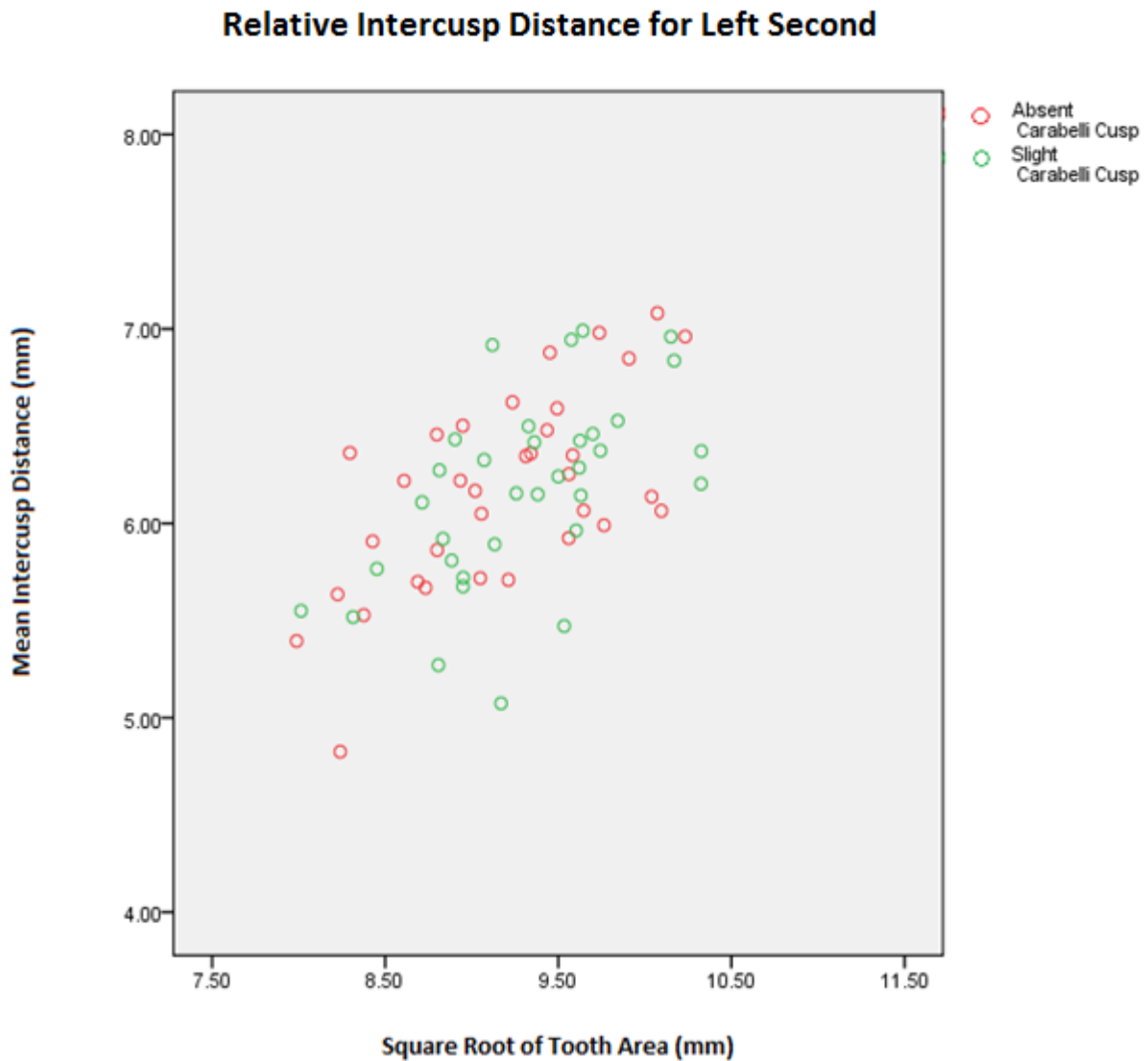


Figure 6. Relative Intercusp Distance Observed in Left Second Molars. The square root of tooth area in mm (absolute tooth area) is graphed against the mean intercusp distance in mm. The molars are divided into two categories: absent consisting of ASU grade 0, slight consisting of ASU grades 1-4.

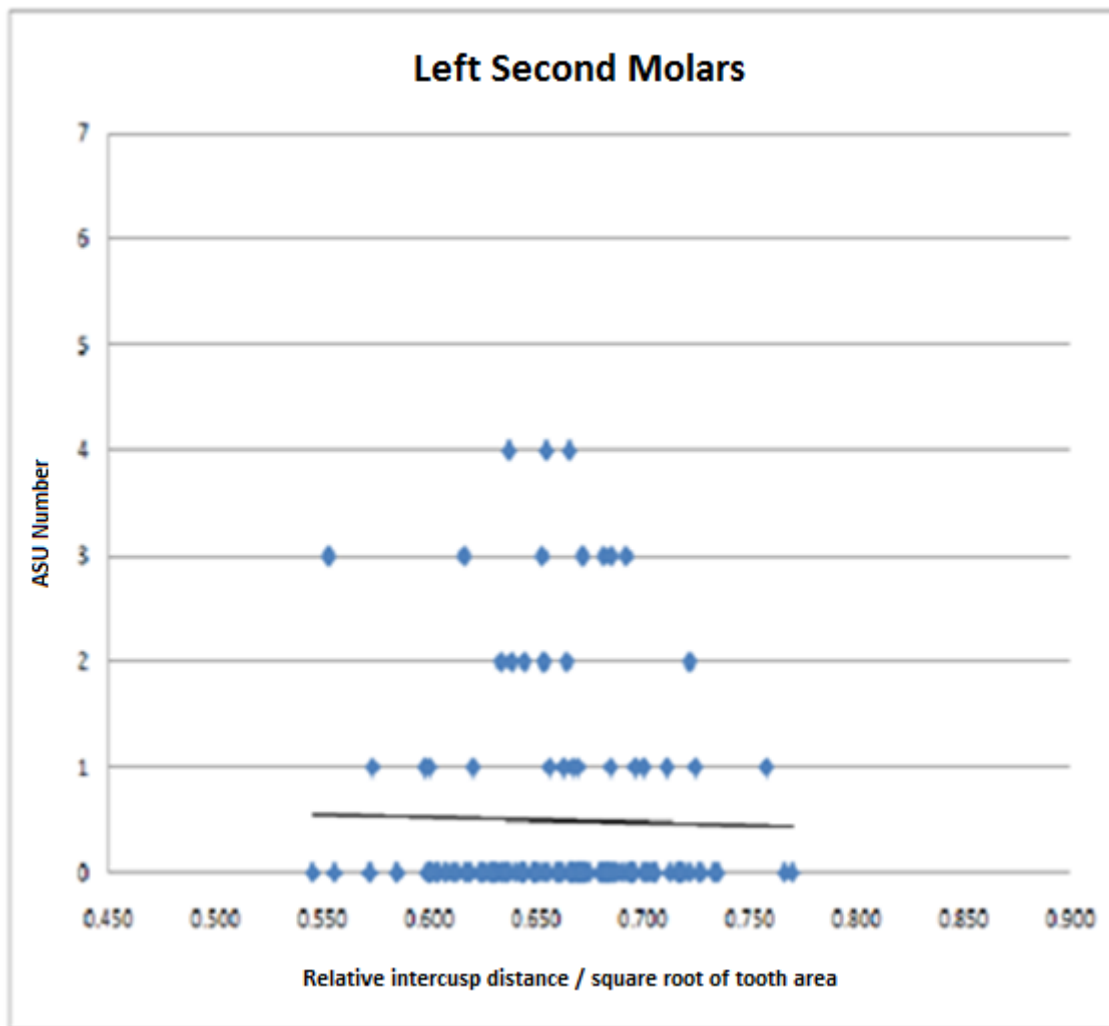


Figure 7. Carabelli Expression Observed in Regard to Relative Intercusp Distance for First and Second Molars Carabelli feature expression is not significantly correlated with relative intercusp distance in second molars ($\tau = 1.279$, $p = 0.2020$, $df = 280$), however, the sample lacks teeth with fully formed Carabelli cusps.

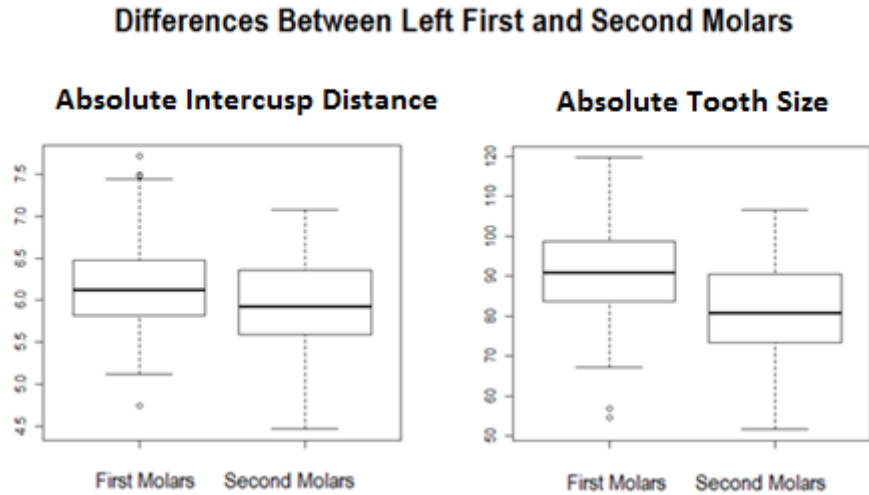


Figure 8. Differences Seen Between First and Second Molars in Mean Intercusp Distance and Absolute Tooth Size Absolute mean intercusp distance ($t = 2.95$, $p = 0.0037$, $df = 131$), absolute tooth size ($t = 5.5297$, $p = 0.0001$, $df = 131$).

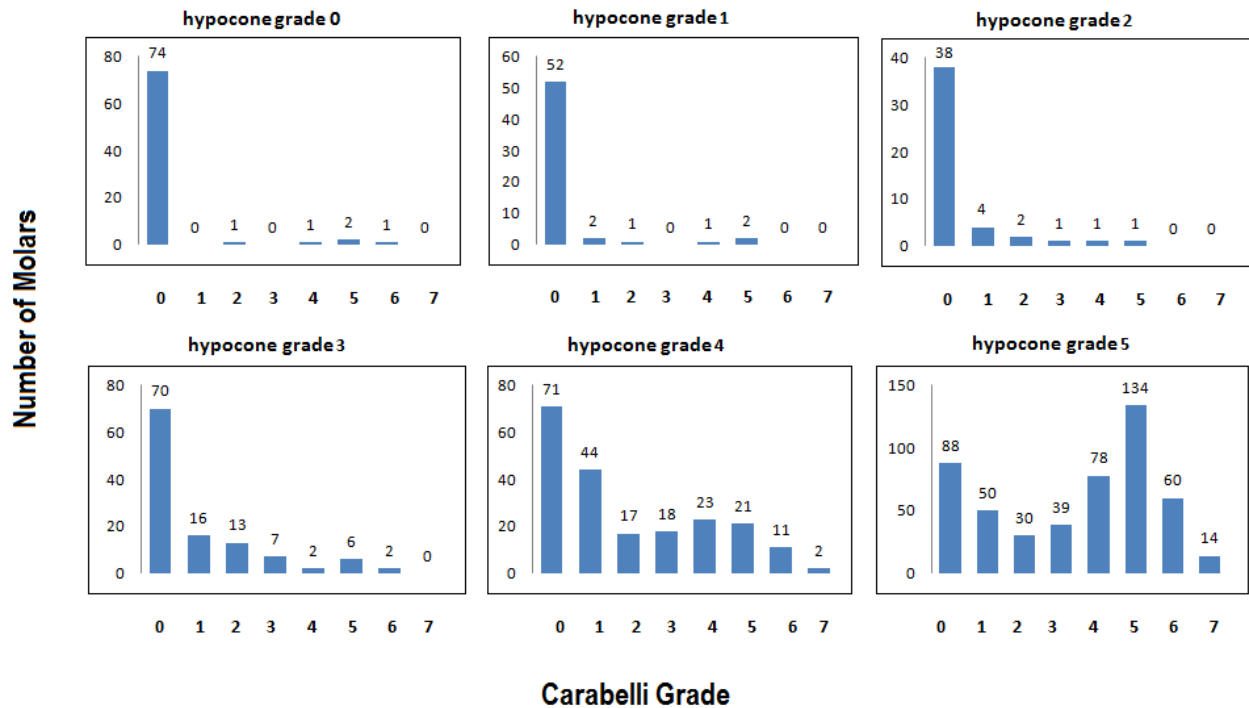


Figure 9. Number of Molars per Carabelli Feature Grade across Hypocone Grades. Each box represents all of the teeth that fall into a specific hypocone grade. The X-axis represents Carabelli Grades 0-7. The Y-axis represents the heights of the bars which indicate the number of molars in each category.

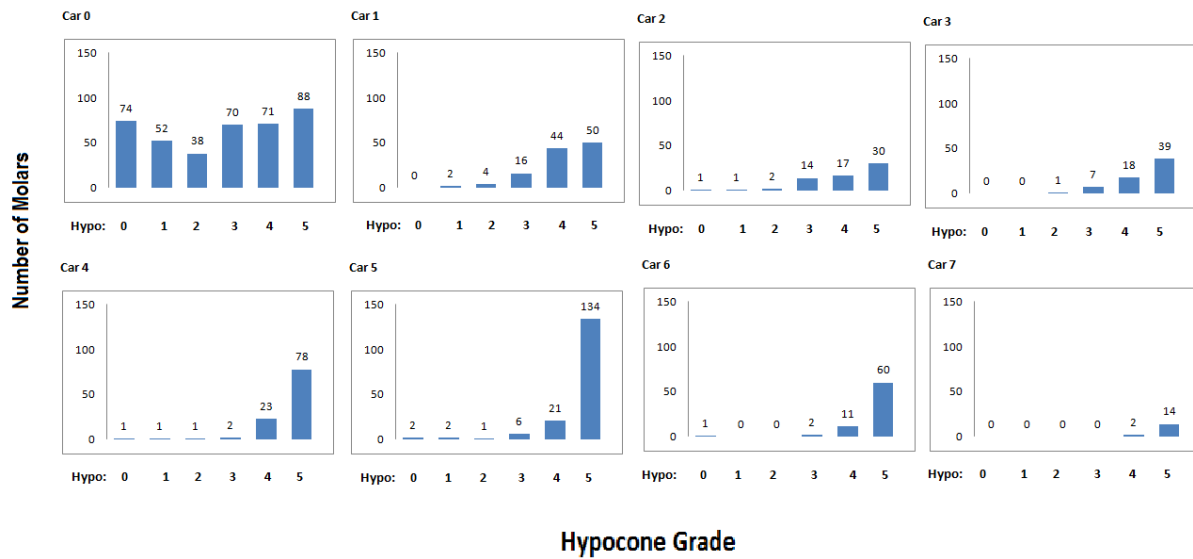


Figure 10. Number of Molars per Hypocone Grade across Carabelli Feature Grades Each box represents all of the teeth that fall into a specific Carabelli feature grade. The X-axis represents Hypocone Grades 0-5. The Y-axis represents the heights of the bars which indicate the number of molars in each category.

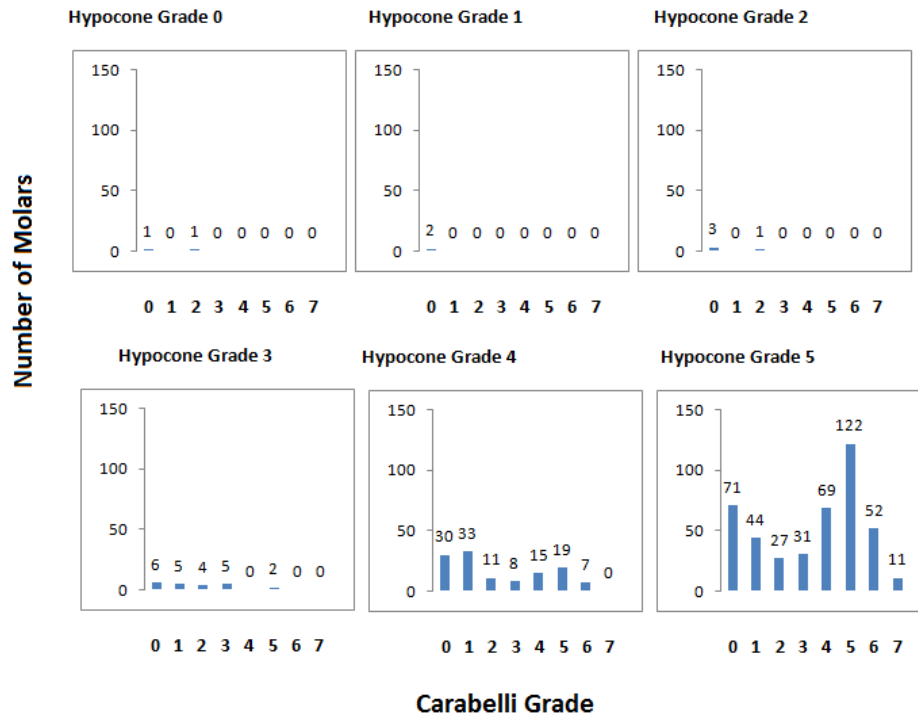


Figure 11. Number of Molars per Carabelli Feature Grade across Hypocone Grades for First Molars. Each box represents all of the teeth that fall into a specific hypocone grade. The X-axis represents Carabelli Grades 0-7. The Y-axis represents the heights of the bars which indicate the number of molars in each category.

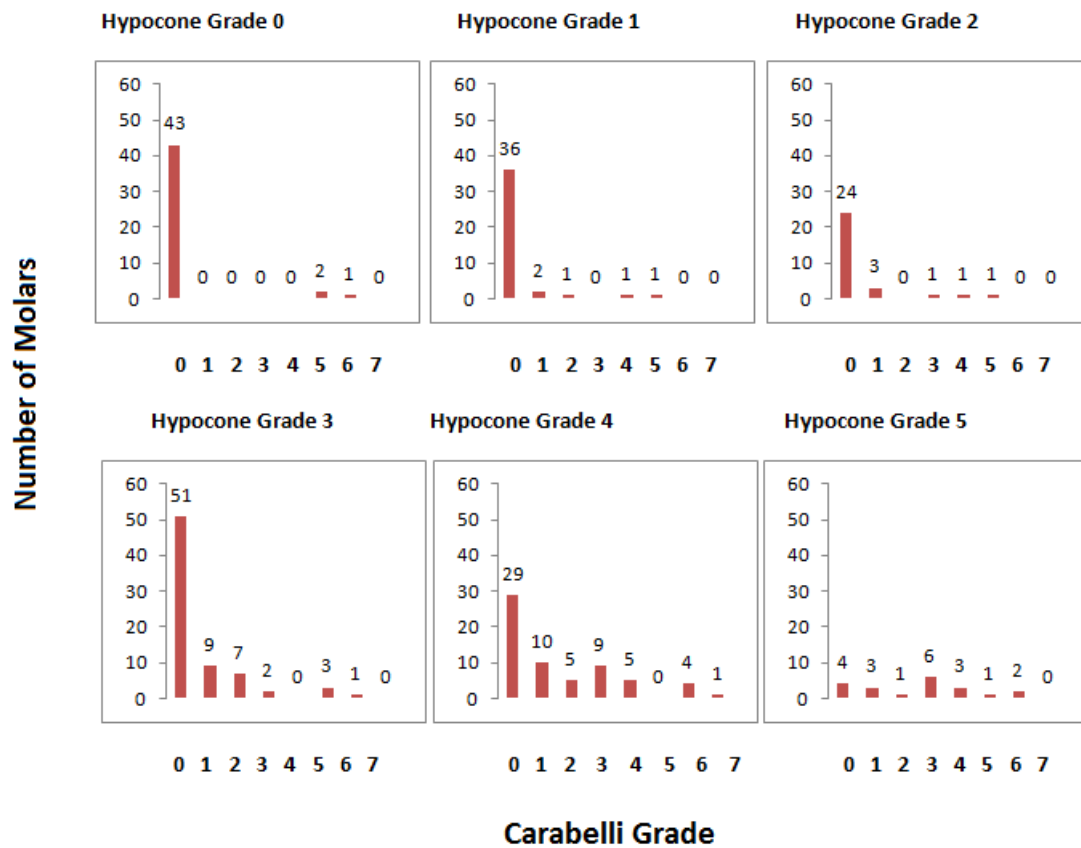


Figure 12. Number of Molars per Carabelli Feature Grade across Hypocone Grades for Second Molars. Each box represents all of the teeth that fall into a specific hypocone grade. The X-axis represents Carabelli Grades 0-7. The Y-axis represents the heights of the bars which indicate the number of molars in each category.

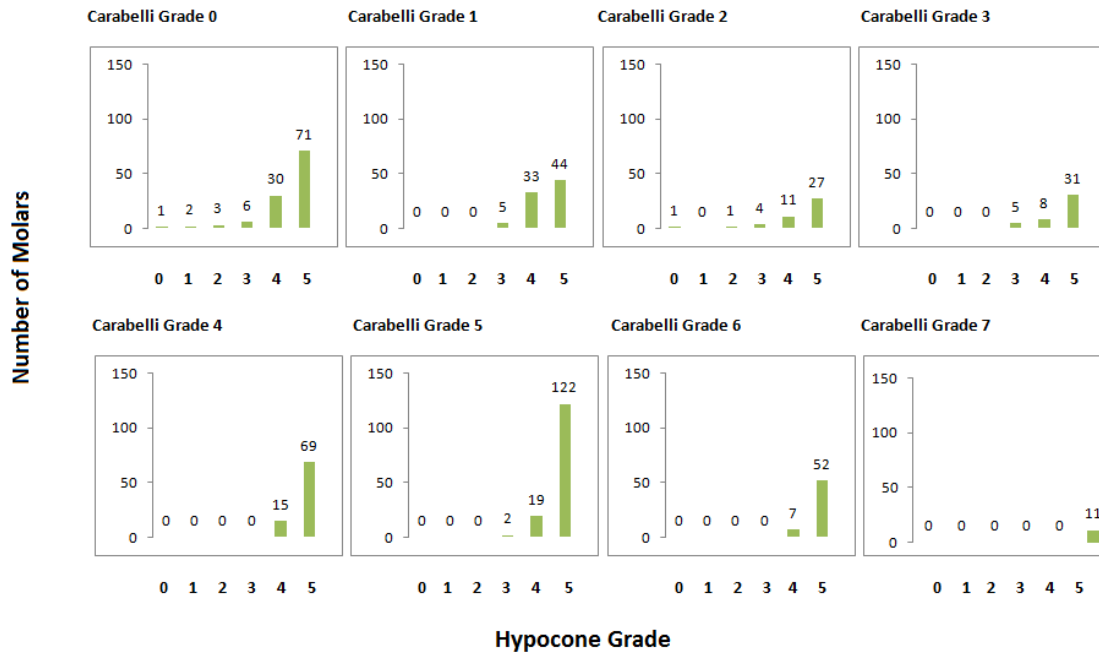


Figure 13. Number of Molars per Hypocone Grade across Carabelli Feature Grades for First Molars Each box represents all of the teeth that fall into a specific Carabelli feature grade. The X-axis represents Hypocone Grades 0-5. The Y-axis represents the heights of the bars which indicate the number of molars in each category.

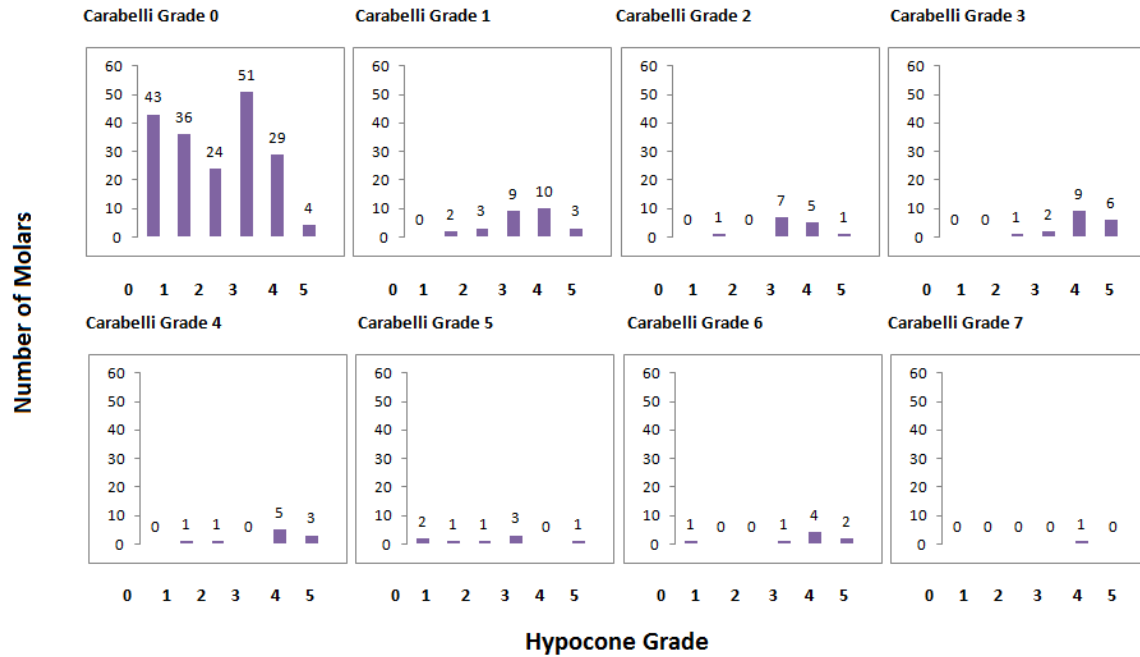


Figure 14. Number of Molars per Hypocone Grade across Carabelli Feature Grades for Second Molars. Each box represents all of the teeth that fall into a specific Carabelli feature grade. The X-axis represents Hypocone Grades 0-5. The Y-axis represents the heights of the bars which indicate the number of molars in each category.

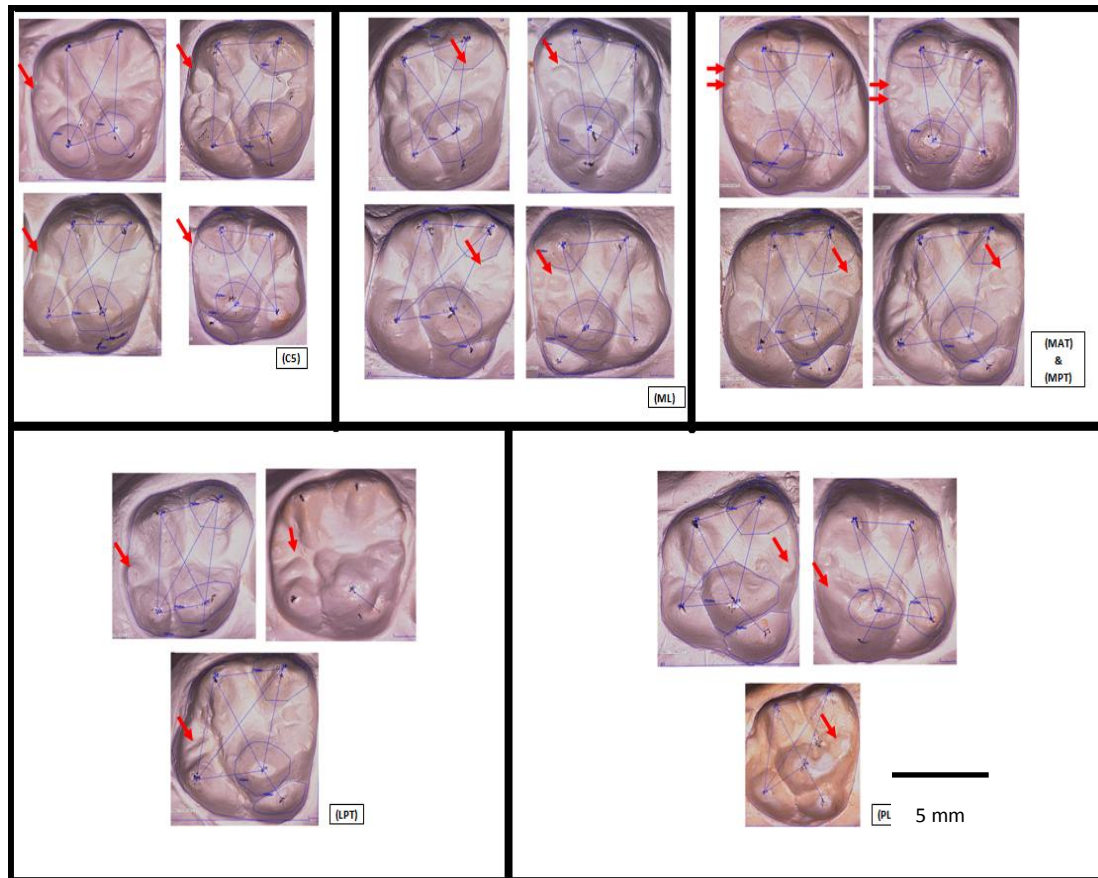


Figure 15. Examples of the Accessory Cusps. Clockwise from the upper left box. (C5) Different Cusp 5s are indicated in varying degrees of development. (ML) Metaconules are indicated in varying degrees of development. (MAT and MPT) Mesial accessory tubercles and mesial paracone tubercles are indicated in varying degrees of development. (LPT) Lingual paracone tubercles are indicated in varying degrees of development. (PL) Protoconules are indicated in varying degrees of development.

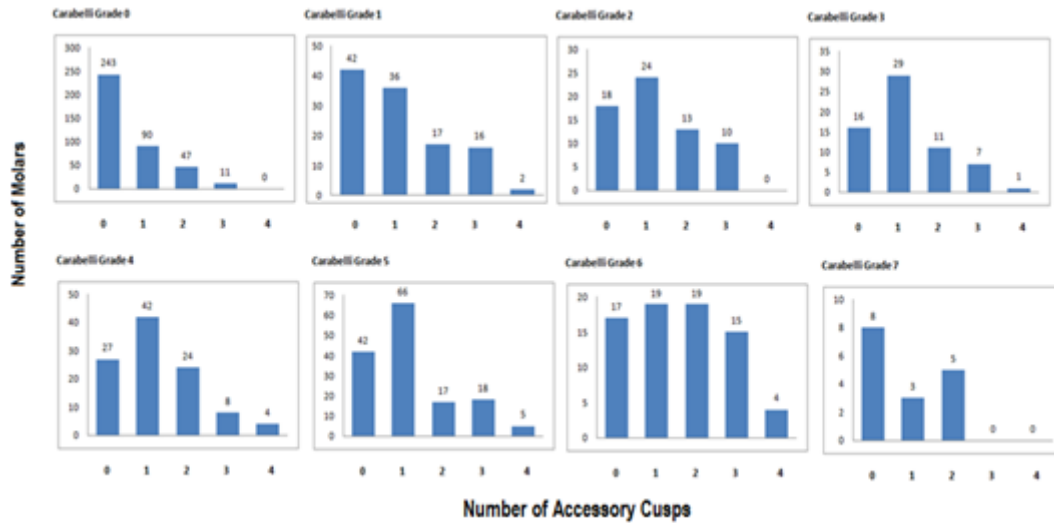


Figure 16. Number of Accessory Cusps vs. Number of Teeth per each Carabelli Feature Grade. Each box represents a different Carabelli feature grade. The X-axis represents the number of accessory cusps expressed on each molar. The height of the bar represents the number of molars present in each category.

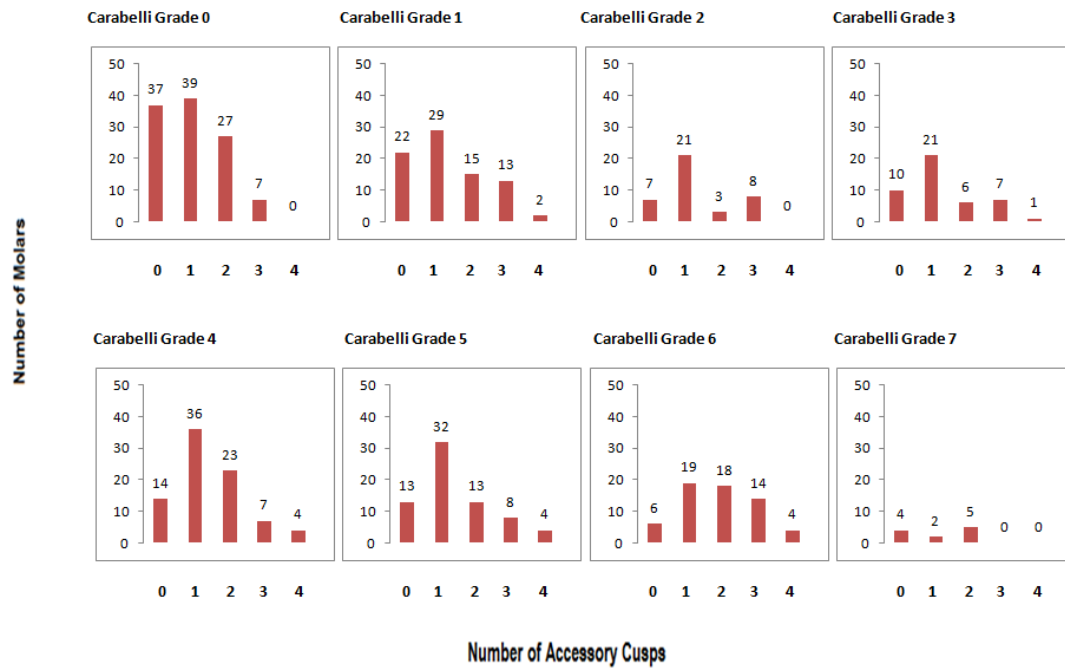


Figure 17. Number of Accessory Cusps vs. Number of Teeth per each Carabelli Grade for First Molars. Each box represents a different Carabelli feature grade. The X-axis represents the number of accessory cusps expressed on each molar. The height of the bar represents the number of molars present in each category.

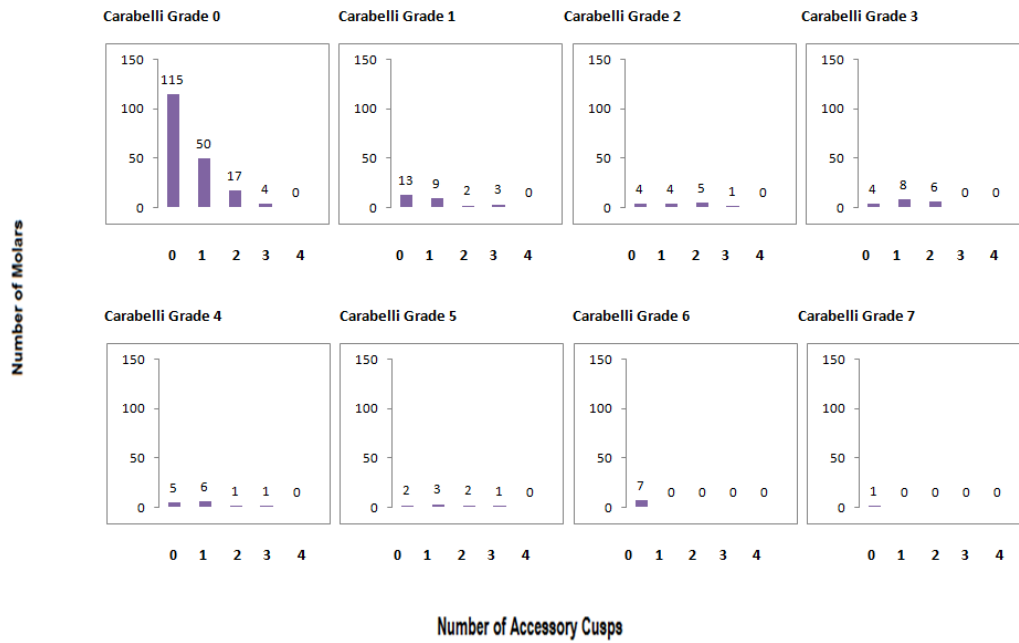


Figure 18. Number of Accessory Cusps vs. Number of Teeth per each Carabelli Grade for Second Molars Each box represents a different Carabelli feature grade. The X-axis represents the number of accessory cusps expressed on each molar. The height of the bar represents the number of molars present in each category.

Measurement	MS Among	MS Within	s2 among ¹	ME (%) ²
Tooth Area	61.0963	2.0616	20.3654	9.19%
Carabelli Area	3.6140	0.0943	1.1732	7.43%
Paracone-Protocone	0.2281	0.0079	0.0734	9.72%
Paracone-Metacone	0.2754	0.0275	0.0826	24.98%
Protocone-Metacone	1.1507	0.0139	0.3789	3.54%
Paracone-Hypocone	0.2654	0.0178	0.0825	17.75%
Protocone-Hypocone	0.5167	0.0410	0.1586	20.54%
Metacone-Hypocone	0.4737	0.0450	0.1429	23.95%

Table 1. Measurement Error. Measurement Error (ME) as a percentage of total among and within individual variation, derived from a Model II ANOVA using three repeated measurements per individual in a subsample of 20 teeth. ¹Estimated as $(MS_{\text{among}} - MS_{\text{within}}) / 4$ measurements per specimen. ²Estimated as $100 \times [(s2_{\text{within}} + s2_{\text{among}})]$, where $s2_{\text{within}} = MS_{\text{within}}$.

Carabelli Grade	Mean ICD (mm)	Mean Square Root Tooth Area	N
LM1 Present (ASU 5-7)	5.670	9.673	87
LM1 Absent (ASU 0)	6.604	9.296	44
LM2 Present (ASU 4-5)	5.973	9.184	12
LM2 Absent (ASU 0)	5.898	8.875	92

Table 2. . Comparisons of Mean Intercusp Distance and Tooth Size to Carabelli feature Expression. Individual comparisons of mean intercuspal distance (mm) and square root of tooth area (mm) in left upper molars to Carabelli feature development (ASU number).

Comparison	Odds	Intercepts	LL(H1) ¹	LL(H0) ²	G ³	Coefficient	P-value
Left First Molars: Number of Accessory cusps and Carabelli Grade	1.125	-0.920	-401.302	-404.515	5.825	0.118	<0.025
		0.871					
		2.068					
		3.947					
Right First Molars: Number of Accessory cusps and Carabelli Grade	1.114	-0.870	-404.275	-404.567	4.584	0.108	<0.05
		0.819					
		2.113					
		3.930					
Left Second Molars: Number of Accessory cusps and Carabelli Grade	1.284	0.377	-156.737	-160.021	6.568	0.250	<0.025
		1.845					
		3.139					

Table 3. Proportional Odds Logistic Regression of Carabelli Expression. Assessed through two typological schemes and treated as an ordered categorical variable. ¹LL(H1) = log-likelihood of estimated model. ²LL(H0) = log-likelihood of null model. ³G = likelihood ratio or -2(LL(H0)-LL(H1)), in each case greater than the critical value of Chi-square at 1 degree of freedom.

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